



Hyundai Hope on Wheels Hyundai Scholar Research

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Background and Significance

Acute Myeloid Leukemia (AML) is a disorder of normal blood cell development. Approximately one thousand children are diagnosed with AML each year in the United States. Their survival using conventional therapies hovers near 50%, which lags considerably behind survival for acute lymphoid leukemia and has changed only modestly in the last decade. This outcome is clearly unacceptable, and reinforces the need for both new insights into AML pathogenesis and new therapeutic targets. Because AML occurs when mechanisms of normal blood cell development are altered, we must better understand these mechanisms, how they are altered in AML and how we can overcome these alterations to improve outcomes for our patients.

At the heart of AML development are mutations in genes that direct hematopoiesis, an exquisitely regulated and flexible process whereby hematopoietic stem and progenitor cells produce more mature cells found in our peripheral blood. The most commonly observed chromosomal translocations seen in AML involve the RUNX1 locus and genes that encode members of the myeloid translocation gene (MTG) family of proteins. These translocations encode fusion proteins composed of RUNX1 and MTG domains that can, at once, impair hematopoietic development, enhance hematopoietic stem and progenitor cell self-renewal capacity, and antagonize the machinery that corrects new mutations as they arise. From this fertile ground, a fully malignant leukemia cell can emerge. In our laboratory, we are working to understand how MTG proteins and their proleukemic derivatives influence the machinery governing hematopoietic stem and progenitor cell self-renewal and how these relationships are regulated. We believe this will lead us to novel targets for therapeutic development that improve

survival for patients with AML. Without the generous support of the Hyundai Hope On Wheels program, our shared mission to cure every pediatric cancer patient could not be realized.

Preliminary Studies

We have found that MTG family proteins and the proleukemic fusion proteins derived from them, interact with core elements of the Notch transcription complex (NTC). The NTC is minimally composed of the nuclear protein CSL, the intracellular domain of a Notch receptor (N-ICD), of which there are four, and the transcriptional co-activator protein Mastermind-like (MAML). Together, this ternary complex stimulates the expression of Notch target genes to control cell fate specification. MTG proteins and their derivatives interact with CSL and each of the four N-ICD proteins. Using an extensive panel of MTG16 mutants, we have mapped the N-ICD binding site to the MTG16 N-terminus and the CSL binding site to a conserved C-terminal motif. MTG proteins and the RUNX1-MTG fusion proteins alter transcription driven by the promoter of the Notch target gene HES1, while Notch-dependent differentiation of hematopoietic progenitors from MTG16 *-/-* mice is impaired *in vitro*. In extending these observations, we have identified a site of phosphorylation in MTG16 that impairs its interaction with the N-ICD. This site is absolutely conserved among MTG family proteins, is retained in the RUNX1-MTG fusion proteins, and may provide a means of regulating their relationships with the NTC. As such, the kinase(s) responsible are candidate regulators of this relationship as well. We hypothesize that the MTG—Notch relationship is fundamental to normal and malignant hematopoiesis. Our goals are to define the role of Notch transcription complex in AML involving MTG mutations and to identify regulators of the MTG—Notch relationship.

Specific Aim #1: Define the role of the NTC to RUNX1-MTG-mediated leukemia.

Experimental Design: We will employ *ex vivo* and *in vivo* assays of hematopoietic progenitors for immortalizing and leukemic potential following retroviral transduction with RUNX1-MTG constructs deficient for binding core NTC components. In reciprocal studies in t(8;21) myeloid leukemia cell lines, we will disrupt NTC—RUNX1-MTG interactions by expressing isolated binding domains for NTC components or abolish their expression using shRNA strategies, then examine growth and differentiation outcomes.

1. Hematopoietic progenitor immortalization. We will transduce hematopoietic progenitors with recombinant retroviruses expressing RUNX1-MTGs, their derivatives or empty vector and a puromycin resistance cassette, followed by selection, serial replating and terminal analyses for lineage specification.
2. RUNX1-MTG—Flt-3 ITD cooperation in hematopoietic transformation: Flt-3 ITD cooperates with RUNX1-MTG8 to transform hematopoietic progenitors. Using our RUNX1-MTG mutants deficient in NTC component interactions, we will assess the need for CSL and N-ICD binding for the transformed phenotype either with or without Flt-3 ITD. Also, our S189 and S216 substitution mutants will begin to address regulation of the RUNX1-MTG—Notch relationship in leukemogenesis.
3. NTC component roles in leukemia cells with t(8;21) translocations: We will utilize lentiviral-based shRNA strategies to abrogate expression of Notch1, CSL and MAML in myeloid leukemia cell lines Kasumi-1 and SKNO-1, which harbor the t(8;21) translocation, then evaluate clonogenicity, cell cycle progression and terminal differentiation. To disrupt CSL or N-ICD interactions with RUNX1-MTG8, we will express their binding domains from MTG16 in these cell lines, then monitor growth and differentiation as above.
4. RUNX1-MTG—Notch relationship in leukemia pathogenesis: We will perform transplantation assays using CD45.1⁺ hematopoietic progenitors transduced with RUNX1-MTGs, their N-ICD and CSL binding deficient derivatives or vector control, with or without Flt3-ITD. After appropriate selection and passage, infected cells will be injected with a radioprotective cell dose from CD45.2⁺ donor mice into lethally irradiated, CD45.2⁺ C57BL/6 recipients. Peripheral blood counts and lineage allocation will be monitored regularly. Moribund mice will be sacrificed, tissues collected for analysis.

Specific Aim #2: Define RUNX1-MTGs relationship to NTC components and identify kinase(s) that modify serine-189/216

Experimental Design: RUNX1-MTG8 binds both CSL and N-ICD, but whether binding is mutually exclusive or reflects a common ternary complex is unknown. Additionally, mechanistic insights into Notch target gene activation by RUNX1-MTG proteins are found in a thorough understanding of these interactions. We will utilize “bridging” co-precipitation, co-sedimentation in sucrose density gradients, gel permeation chromatography and chromatin immunoprecipitation strategies to gain additional insights into the relationship between RUNX1-MTGs and the NTC. Additionally, we propose a kinome wide screen for modifiers of S189/216 in RUNX1-MTG8 and RUNX1-MTG16, respectively.

1. *Bridging Co-precipitation*: This approach exploits a common binding protein for two factors whose direct interaction has been abolished by mutation. CSL, N1-ICD, MAML and RUNX1-MTGs or mutants with selected binding deficiencies will be co-expressed in combinations and immune complexes directed toward NTC components will be analyzed for the presence of RUNX1-MTG or its derivatives, as well as other complex components, by immunoblotting.
2. *Co-purification of RUNX1-MTGs with NTC components*: To validate co-precipitation data, co-sedimentation in rate zonal sucrose density gradient centrifugation and gel permeation chromatography will be performed on nuclei harvested from COS7L cells expressing Notch transcription complex components and RUNX1-MTGs or binding deficient derivatives.
3. *Chromatin immunoprecipitation (ChIP)*: Dynamic changes in the Notch transcription complex in the context of inducible RUNX1-MTG expression will be assessed in HL-60 cells in both the absence and presence of Notch activating stimuli from co-cultured OP9-DL1 cells by ChIP. Immune precipitation will be directed at RUNX1-MTGs or NTC components through a time course after Notch activation, followed by amplification of the Notch responsive promoters HES1 and Hey1 in immune complexes.
4. *Kinome screen for S189 directed kinases*: We have devised a whole kinome screening strategy for kinases that modify MTG proteins and their derivatives. *In vitro* kinase assays will be performed using residues 1-269 of RUNX1-MTG8, which contains S189 and flanking sequence to provide structural context. Phospho-S189 will be identified in immunoblotting. This strategy is applicable to RUNX1-MTG16 and other MTG proteins as well.